Full text provided by www.sciencedirect.com
SCIENCE DIRECT®

ELSEVIER

Stem cell regulation in the *Arabidopsis* shoot apical meristem

Leor Williams and Jennifer C Fletcher

The aerial structure of higher plants is generated dynamically throughout the life cycle through the activity of stem cells that are located at the growing shoot tip, the apical meristem. The stem cells continuously divide to renew themselves and provide cells for leaf, stem and flower formation. Stem cell maintenance is governed by intercellular communication between the apical stem cells and the underlying organizing centre. Recent advances have been made in understanding the mechanisms that induce shoot stem cell identity, and that control the position and size of the organizing centre. Elements such as chromatin remodeling factors, transcription factors and microRNAs are newly implicated in these regulatory processes. These advances provide a framework for our understanding of how signals are integrated to specify and position the stem cell niche in the shoot apical meristem.

Addresses
USDA-ARS Plant Gene Expression Center, 800 Buchanan Street, Albany, California 94710, USA and Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, California 94720, USA

Corresponding author: Fletcher, Jennifer C (fletcher@nature.berkeley.edu)

Current Opinion in Plant Biology 2005, **8**:582–586

This review comes from a themed issue on
Cell biology
Edited by Patricia C Zambryski and Karl Oparka

Available online 23rd September 2005

1369-5266/$ – see front matter
© 2005 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.pbi.2005.09.010

Introduction

Self-renewing stem cell reservoirs that are located at the very apex of the shoot apical meristem (SAM) are the key to continuous aerial growth and development of higher plants. These stem cells divide slowly and produce daughter cells that can have two types of fate. Those daughter cells that stay in the center remain stem cells, whereas others are continuously displaced from the center outward towards the periphery or downward into the interior. These cells divide more rapidly than the central stem cells [1] and provide the founder cells for the formation of either lateral organs or the main stem. The number of stem cells remains constant despite the continuous departure of their daughters into initiating lateral organs, indicating that the recruitment of cells into new organs is precisely balanced by the formation of new stem cell derivatives. Classic physiological and modern molecular experiments have shown that intrinsic intercellular signaling, rather than genetically predetermined cell-fate specification, controls the organization and activity of the SAM.

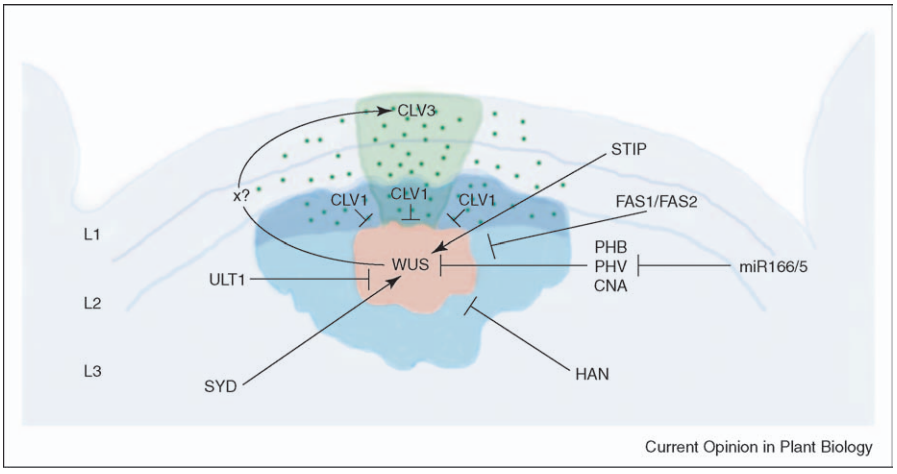
A key question in meristem biology is how different signals integrate to specify and maintain the correct position and number of stem cells and to coordinate proper meristem function. SAM maintenance in *Arabidopsis* involves a spatial negative feedback loop between the secreted signaling molecule *CLAVATA*3 (*CLV*3) and the homeodomain protein *WUSCHEL* (*WUS*) (reviewed in [2,3]). Although there is much evidence supporting the CLV3–WUS feedback loop, there are many open questions regarding the dynamics of this network. In this review, we highlight some of these questions and describe recent progress that provides a foundation for addressing them.

Shoot meristem maintenance by the CLV–WUS feedback loop

Stem cell identity is specified by signaling from a small group of cells underneath the stem cell region, termed the organizing centre (OC). These cells express *WUS*, which encodes a member of the WOX family of putative homeodomain transcription factors [4]. Loss of *WUS* function results in the mis-specification of stem cells and the premature termination of the SAM, thus *WUS* is essential for maintenance of the stem cell reservoir [5]. Ectopic *WUS* expression is sufficient to induce ectopic stem cell fate [6], indicating that the *WUS* expression domain must be tightly controlled and that the *WUS*-mediated inductive signal must be targeted specifically to the apical cells and kept away from other cells to maintain the correct position and number of stem cells. The stem cells signal back to the OC via the *CLV* signaling pathway. CLV3 is a small, secreted polypeptide that is produced by the stem cells [7,8••] and that is postulated to interact with the CLV1–CLV2 receptor complex in the underlying cells. Activation of the CLV complex initiates downstream signaling events that limit the size of the *WUS* expression domain [9]. In turn, *WUS* activity in the OC specifies the overlying neighbors as stem cells and induces the expression of *CLV3*. Through this negative regulatory feedback loop between the stem cells and the cells of the underlying OC (Figure 1), any increase or decrease in stem cell number results in a corresponding change in *CLV3* transcript levels and an immediate adjustment of *WUS* expression.

The stem cell homeostasis mediated by the *CLV–WUS* feedback loop is highlighted by the striking differences in

Figure 1



Molecular mechanisms that regulate stem cell fate in the *Arabidopsis* shoot apical meristem. Stem cells are located in the outermost cell layers of the center of the meristem (green). This population is maintained by an unknown signal (X?) that is generated by the underlying *WUS*-expressing cells in the organizing center (OC; orange). The stem cells express *CLV3*, which encodes a secreted signaling molecule (dark green dots) that moves laterally and downward to the underlying cell layers. CLV3 activates a signaling pathway that is mediated by the CLV1 receptor kinase (blue), which limits the size of the *WUS*-expressing OC. The putative transcriptional regulators ULT1 and HAN restrict the expansion of the *WUS*-expression domain through CLV-independent pathways. The class III HD-ZIP proteins PHB, PHV and CNA negatively regulate the level of *WUS* mRNA that is transcribed by the OC cells, and are themselves subject to negative regulation by microRNA166/5. STIP positively regulates *WUS* expression in the OC and is subject to negative regulation by the *CLV* genes. The FAS1/FAS2 and SYD proteins act through chromatin-mediated processes to prevent ectopic *WUS* activation by cells outside the OC and to maintain *WUS* expression at high levels within the OC, respectively.

the effects of *CLV3* transgene activity depending on the promoter used. Lenhard and Laux [8••] showed that the expression of one copy of the *CLV3* gene under the control of the *ATML1* promoter, which is independent of *WUS* activity, was sufficient to cause SAM termination. By contrast, up to five copies of *CLV3* under the control of the *CLV3* promoter are tolerated and merely cause a reduction in overall meristem size. In these *pCLV3*::(*CLV3*)*5* expressing plants, the high level of *CLV3* leads to downregulation of *WUS* expression, which in turn causes a reduction in the transgene expression level, allowing a new balance between *WUS* and *CLV3* activities to be established and a smaller, but functional, meristem to be maintained.

Induction of shoot stem cell identity

Genetic and molecular data indicate that WUS acts in a non-cell-autonomous fashion [10,11], either by moving outward from the cells in which it is transcribed or by activating an intercellular signal. The non-autonomous activity of WUS raises the question of how WUS-mediated induction of stem cell fate is restricted to the cells above the OC rather than those surrounding it. Is the signal directed solely to the correct cells at the apex or, alternatively, is it released to all the surroundings cells but only those cells that are 'stem cell competent' are capable of perceiving it?

Several recent studies provide evidence supporting a 'stem cell competency' theory. Seedlings that express a *pCLV3*::*WUS* transgene developed a large apical dome [12]. The stem cell marker *CLV3* was found only in the three apical cell layers throughout the dome and not in the deeper region of the meristem, indicating that only the most apical cells in the meristem are specified as stem cells. Furthermore, Gallois *et al.* [13••] studied the effects of *WUS* activity outside the shoots by inducing *WUS* transcription in roots using a Cre-*lox*P-based mosaic expression system. When *WUS* was expressed throughout the roots, the phenotypic effects of stem cell formation and *CLV3* activation were seen only at the primary and lateral root tips, demonstrating that although the *WUS* mRNA was present in every cell, the response to *WUS* was restricted to the meristematic regions. These results suggest that only certain 'competent cells' are able to perceive and/or respond to the *WUS*-derived signal. Such cells might, for example, have a particular chromatin configuration or express specific receptor(s) that enable them to respond to a signaling molecule activated by *WUS*.

The Gallois *et al.* [13••] study also addressed the question of whether *WUS* might protect stem cell identity by antagonizing differentiation signals, and whether this antagonism is directed against signals that promote shoot cell fates or against a general differentiation signal. Interestingly, they found that the expression of *WUS* in the root induces the expression of shoot stem cell markers and the formation of shoot lateral organs. The results imply that *WUS* does not simply establish naïve stem cells that require

input from surrounding tissues to develop as shoot cells, but instead establishes cells with the intrinsic potential to generate shoot tissues. The conclusion is that *WUS* alone, without additional cues, is sufficient to activate a gene expression program that specifies shoot stem cell identity.

Regulatory elements in the *WUS* promoter

WUS plays a key role in the positioning of the stem cell niche, but until recently, little was known about the regulatory circuits that control its highly restricted expression in the OC. The β-glucuronidase (GUS) activity in the meristems of plants carrying a *WUS*::*GUS* promoter construct shows the same pattern as that described for *WUS* mRNA [10], indicating that the regulatory sequences that control the boundaries of *WUS* expression are present as *cis* elements in the *WUS* promoter. An interesting new finding from analysis of the *WUS* promoter is that a 57-bp regulatory region located 529 bp upstream of the start codon is sufficient to provide all the information required for *WUS* transcription in the SAM [14••]. The promoter activity can be assigned to two adjacent short sequence motifs (RE1 and RE2) within the 57-bp region. Thus, the diverse regulatory pathways that control the *WUS* expression converge at two short sequence elements, suggesting that the integration of regulatory signals might take place at the level of a central *trans*-activating complex. A tetramer of the RE1 and RE2 sequences that was cloned upstream of a minimal 35S promoter was sufficient to confer *WUS* expression. The expression levels appeared to be highly dependent on the site of integration within the genome, however, suggesting that the effectiveness of RE1 and RE2 requires a favorable chromatin state. By contrast, no integration site dependency was observed for constructs containing the full-length *WUS* promoter, raising the question of whether other regulatory regions in the promoter act in chromatin organization at the *WUS* locus.

Chromatin regulation of *WUS* in the SAM

Cellular chromatin organization is constantly remodeled during the activation and repression of specific sets of genes that are required for development. A range of chromatin remodeling factors are involved in controlling meristem function and organogenesis, mainly by regulating the spatial and/or temporal expression of key transcription-factor-encoding genes [15]. That *WUS* is one such target is shown by the variegated and ectopic expression of *WUS* observed in plants carrying loss-of-function mutations in the *FASCIATA1* (*FAS1*) or *FAS2* genes, which encode subunits of CHROMATIN ASSEMBLY FACTOR-1 (CAF-1) [16]. BRUSHY1 (BRU1), a protein involved in the post-replicative stabilization of chromatin structure, has been associated with positive regulation of the *WUS* expression domain [17].

A recent study shows that *WUS* is a direct target of the chromatin remodeling factor SPLAYED (SYD) [18••], a SNF2 class ATPase from a family known to facilitate transcription by creating a DNA template that is accessible to the general transcription apparatus [19]. Adult *syd* plants undergo premature termination of the SAM, correlating with a reduction in the message levels of *WUS* and *CLV3*. Chromatin immunoprecipitation experiments demonstrated that SYD is specifically recruited to the proximal promoter region of the *WUS* locus. Thus SYD is a direct and specific positive upstream regulator of *WUS* expression that is required to maintain proper *WUS* transcript levels in its normal expression domain. These data provoke the challenging question: what are the mechanisms through which chromatin factors integrate developmental signals to regulate meristem function?

Transcriptional regulation of *WUS* expression

The ability of WUS to induce stem cell formation and the restriction of *WUS* mRNA to a small group of cells in the OC illustrates the requirement for tight control of *WUS* expression at the transcriptional level. Several new studies have addressed some longstanding questions about *WUS* regulation, including how *WUS* transcription is activated at the center of the SAM, how the expression domain is stably maintained and restricted to a small region of cells within the proliferating shoot apex, and how the level of *WUS* expression is regulated.

One regulator of *WUS* that acts very early in *Arabidopsis* development is the GATA-3-like transcription factor HANABA TARANU (HAN), which is expressed at the SAM boundary and appears to be involved in controlling cell proliferation and differentiation [20•]. The *WUS* expression domain of *han* mutants begins to expand early in embryogenesis, before *CLV3* and *CLV1* expression is initiated, suggesting that HAN is required to control the number of *WUS* expressing cells and to position these cells correctly. The ULTRAPETALA1 (ULT1) putative transcriptional regulatory protein is implicated as a spatial regulator of *WUS* expression during later stages of development [21]. Mutations in the *ULT1* gene cause the enlargement of inflorescence and floral meristems and the lateral expansion of the *WUS* expression domain, indicating that *ULT1* is a key negative regulator of stem cell accumulation after the floral transition. Double mutants generated between *ult1* and *clv* alleles reveal that these genes have overlapping functions as negative regulators of the *WUS*-expressing OC but act in separate genetic pathways [22].

Another factor that maintains *WUS* expression in the OC is STIMPY/WOX9 (STIP), a *WUS*-related homeobox-containing protein that was identified in an activation-tagging screen [23•]. The SAM of *stip* mutants fails to grow into a dome-shaped structure and lacks both *CLV3* and *WUS* expression. Interestingly the *stip* mutant phenotype can be fully rescued by adding sucrose, implying that STIP maintains growth by stimulating the entry of

SAM cells into the cell cycle. Loss of STIP function completely suppresses the *clv3* phenotype whereas overexpression enhances it, consistent with *STIP* acting in the *CLV–WUS* pathway. The authors propose a model in which *STIP* positively regulates the expression of *WUS* in the OC and is subject to negative regulation by the *CLV* genes. Other exogenous factors, such as sucrose, also promote *WUS* expression in the SAM by stimulating cell division. These results are the first indication that the nutritional state of the plant can affect meristem activity.

Several recent studies provide evidence for the involvement of class III homeodomain-leucine zipper (HD-ZIP III) transcription factors in *WUS* regulation, and demonstrate that the modulation of *WUS* transcription levels within the OC cells is critical for proper stem cell specification and meristem maintenance. The *CORONA* (*CNA*) gene was identified in a screen for enhancers of the *clv1* meristem phenotype [24], and is proposed to regulate stem cell accumulation in a pathway parallel to that of the *CLV* loci. *CNA* encodes an HD-ZIP III transcription factor, one of five family members that control various aspects of *Arabidopsis* development (reviewed in [25]). Although *cna* single mutants have no meristem defects, the triple mutant of *cna* with *phabulosa* (*phb*) and *phavoluta* (*phv*), two other members of the *HD-ZIP III* gene family, was shown to effectively recreate the *clv* enlarged-SAM phenotype [26]. The involvement of *CNA*, *PHB* and *PHV* in shoot meristem maintenance was also demonstrated by analysis of dominant, gain-of-function *jabba-1D* (*jba-1D*) mutants [27•]. The SAMs of *jba-1D* plants begin to enlarge during embryogenesis, accumulate excess stem cells and ultimately undergo splitting and fasciation. This phenotype requires *WUS* activity and correlates with a dramatic increase in the level of *WUS* transcription in the OC cells. *jba-1D* plants overexpress the microRNA *miR166g*, which causes the loss of *PHB*, *PHV* and *CNA* transcripts in the SAM. From these data it was proposed that, in wildtype plants, *PHB*, *PHV* and *CNA* restrict SAM activity by downregulating *WUS* transcription.

Finally, mathematical modeling techniques are now being applied to describe and test hypotheses about the dynamics of shoot meristem function. Computational modeling tools together with *in vivo* live imaging of the SAM were used to investigate the organization of the *WUS* expression domain [28•]. Jonsson *et al.* [28•] devised a model using a reaction–diffusion mechanism in which an activator molecule induces *WUS* expression. A small repressive signal from the L1 cell layer and the stem is sufficient to place the peak of the activator at the correct position in the OC. This model accurately predicts both the spatial expansion of the *WUS* domain in the absence of CLV3 and the reorganization that occurs in the SAM upon ablation of cells in the OC.

Conclusions and future directions

Recent studies have provided exciting new data that enable us to better understand the molecular mechanisms of stem cell specification and meristem maintenance. Newly identified genes such as *ULT1*, *HAN* and *STIP* add insight into the activities that set the boundaries of the *WUS* expression domain in the OC. Significant progress is also being made to elucidate the factors controlling *WUS* transcription directly at the promoter. Finding essential *cis* elements in the *WUS* promoter together with the first protein shown to bind specifically to the *WUS* promoter, SYD, opens the way to new discoveries on the transcriptional regulation of this stem-cell promoting master gene. The identification of SYD as a positive regulator of *WUS* also demonstrates the importance of chromatin regulators in meristem function. Another major advance is the finding of microRNA involvement in the regulation of stem cell maintenance. *miR166g* might be the first of many small regulatory RNAs that are found to participate in the regulation of SAM activity.

However, there are still many unanswered questions concerning the networks of intercellular and intracellular factors that confer stem cell identity. No genes that are directly regulated by *WUS* have yet been identified, neither has the nature of the inductive signal from the OC to the stem cells been elucidated. Genes that are essential for stem cell function in a cell-autonomous fashion remain to be discovered, as do other intracellular components of the CLV signaling pathway. Answers to these questions may soon be on the horizon as advanced tools, such as laser capture microdissection, microarrays and chromatin immunoprecipitation, are combined with traditional genetic and biochemical methods.

Acknowledgements

The authors acknowledge support for their work on stem cells from the US–Israel Bi-national Agricultural Research and Development Fund (BARD), the National Science Foundation (NSF) and the US Department of Agriculture (USDA).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Reddy GV, Heisler MG, Ehrhardt DW, Meyerowitz EM: **Real-time lineage analysis reveals oriented cell divisions associated with morphogenesis at the shoot apex of *Arabidopsis thaliana***. *Development* 2004, **131**:4225-4237.

2. Baurle I, Laux T: **Apical meristems: the plant's fountain of youth**. *Bioessays* 2003, **25**:961-970.

3. Carles CC, Fletcher JC: **Shoot apical meristem maintenance: the art of a dynamic balance**. *Trends Plant Sci* 2003, **8**:394-401.

4. Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T: **Expression dynamics of *WOX* genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana***. *Development* 2004, **131**:657-668.

5. Laux T, Mayer KF, Berger J, Jürgens G: **The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis***. *Development* 1996, **122**:87-96.

6. Schoof H, Lenhard M, Haecker A, Mayer KF, Jürgens G, Laux T: **The stem cell population of *Arabidopsis* shoot meristems in maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes**. *Cell* 2000, **100**:635-644.

7. Rojo E, Sharma VK, Kovaleva V, Raikhel NV, Fletcher JC: **CLV3 is localized to the extracellular space, where it activates the *Arabidopsis CLAVATA* stem cell signaling pathway**. *Plant Cell* 2002, **14**:969-977.

8. •• Lenhard M, Laux T: **Stem cell homeostasis in the *Arabidopsis* shoot meristem is regulated by intercellular movement of CLAVATA3 and its sequestration by CLAVATA1**. *Development* 2003, **130**:3163-3173.

This study highlights the potential of the dynamic autoregulatory interaction between the stem cells and the OC, which is mediated by the *WUS* and *CLV3* genes, to keep the size of the stem cell population constant. By using different promoters to drive *CLV3* expression, the authors demonstrate that WUS induction of *CLV3* expression is mediated at the level of the *CLV3* promoter itself. This paper also confirms that CLV3 protein moves within the shoot meristem and provides data that suggest that CLV3 movement is limited by the CLV1 receptor.

9. Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R: **Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by CLV3 activity**. *Science* 2000, **289**:617-619.

10. Mayer KF, Schoof H, Haecker A, Lenhard M, Jürgens G, Laux T: **Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem**. *Cell* 1998, **95**:805-815.

11. Gallois JL, Woodward C, Reddy GV, Sablowski R: **Combined SHOOT MERISTEMLESS and WUSCHEL trigger ectopic organogenesis in *Arabidopsis***. *Development* 2002, **129**:3207-3217.

12. Brand U, Grunewald M, Hobe M, Simon R: **Regulation of *CLV3* expression by two homeobox genes in *Arabidopsis***. *Plant Physiol* 2002, **129**:565-575.

13. •• Gallois JL, Nora FR, Mizukami Y, Sablowski R: **WUSCHEL induces shoot stem cell activity and developmental plasticity in the root meristem**. *Genes Dev* 2004, **18**:375-380.

The authors studied the effects of ectopic *WUS* expression outside the shoot. Expression of *WUS* in the root induced ectopic stem cell formation, which without additional cues resulted in the production of shoot lateral organs. This is the first demonstration that the stem cells specified by *WUS* have intrinsic shoot identity.

14. •• Baurle I, Laux T: **Regulation of *WUSCHEL* transcription in the stem cell niche of the *Arabidopsis* shoot meristem**. *Plant Cell* 2005, **17**:2271-2280.

An important and extensive study that analyzes the transcriptional control of *WUS*. This work revealed that a 57-bp regulatory region in the *WUS* promoter provides all of the information required to set the boundaries of *WUS* transcription in the SAM. Two adjacent short sequence motifs within the 57-bp region are sufficient to mimic the native *WUS* mRNA expression pattern. These results demonstrate that the diverse regulatory pathways that control *WUS* expression converge at two short sequence elements, suggesting that a central *trans*-activating complex that binds to these sites might integrate multiple regulatory signals.

15. Guyomarc'h S, Bertrand C, Delarue M, Zhou DX: **Regulation of meristem activity by chromatin remodelling**. *Trends Plant Sci* 2005, **10**:332-338.

16. Kaya H, Shibahara KI, Taoka KI, Iwabuchi M, Stillman B, Araki T: ***FASCIATA* genes for chromatin assembly factor-1 in *Arabidopsis* maintain the cellular organization of apical meristems**. *Cell* 2001, **104**:131-142.

17. Takeda S, Tadele Z, Hofmann I, Probst AV, Angelis KJ, Kaya H, Araki T, Mengiste T, Scheid OM, Shibahara K *et al.*: **BRU1, a novel link between responses to DNA damage and epigenetic gene silencing in *Arabidopsis***. *Genes Dev* 2004, **18**:782-793.

18. •• Kwon CS, Chen C, Wagner D: ***WUSCHEL* is a primary target for transcriptional regulation by SPLAYED in dynamic control of stem cell fate in *Arabidopsis***. *Genes Dev* 2005, **19**:992-1003.

This is a highly significant paper showing that *WUS* is a direct target of the chromatin-remodeling factor SPLAYED (SYD), a SNF2 class ATPase. SYD is shown to be a positive upstream regulator of *WUS* expression that is required for proper *WUS* transcript levels in its normal expression domain, highlighting the importance of chromatin regulation in the control of plant stem cell identity. This paper provides the first evidence of a protein that is specifically recruited to the proximal promoter region of the *WUS* locus.

19. Narlikar GJ, Fan HY, Kingston RE: **Cooperation between complexes that regulate chromatin structure and transcription**. *Cell* 2002, **108**:475-487.

20. • Zhao Y, Medrano L, Ohashi K, Fletcher JC, Yu H, Sakai H, Meyerowitz EM: **HANABA TARANU is a GATA transcription factor that regulates shoot apical meristem and flower development in *Arabidopsis***. *Plant Cell* 2004, **16**:2586-2600.

This work identifies HANABA TARANU (HAN), a GATA-3-like transcription factor that affects both flower and shoot apical meristem function, as a regulator of *WUS* expression. In *han* mutants, the *WUS* expression domain expands early in embryogenesis, indicating that HAN is required to control the number and position of *WUS*-expressing cells. The authors also present data that suggest that HAN is involved in controlling cell proliferation and differentiation.

21. Carles CC, Choffnes-Inada D, Reville K, Lertpiriyapong K, Fletcher JC: ***ULTRAPETALA1* encodes a SAND domain putative transcriptional regulator that controls shoot and floral meristem activity in *Arabidopsis***. *Development* 2005, **132**:897-911.

22. Carles CC, Lertpiriyapong K, Reville K, Fletcher JC: **The *ULTRAPETALA1* gene functions early in *Arabidopsis* development to restrict shoot apical meristem activity and acts through *WUSCHEL* to regulate floral meristem determinacy**. *Genetics* 2004, **167**:1893-1903.

23. • Wu X, Dabi T, Weigel D: **Requirement of homeobox gene *STIMPY/WOX9* for *Arabidopsis* meristem growth and maintenance**. *Curr Biol* 2005, **15**:436-440.

The authors show that STIMPY/WOX9 (STIP) functions as a positive regulator of *WUS* expression in the SAM. *STIP* is shown to maintain *WUS* and *CLV3* expression, and is proposed to act by inducing meristem cells to enter the cell cycle. The *stip* mutant phenotype was fully rescued by adding sucrose, demonstrating that exogenous factors can promote *WUS* expression in the OC by stimulating cell division. These results are the first indication that the nutritional state of the plant can affect meristem activity.

24. Green KA, Prigge MJ, Katzman RB, Clark SE: ***CORONA*, a member of the class III homeodomain leucine zipper gene family in *Arabidopsis*, regulates stem cell specification and organogenesis**. *Plant Cell* 2005, **17**:691-704.

25. Bowman JL, Eshed Y, Baum SF: **Establishment of polarity in angiosperm lateral organs**. *Trends Genet* 2002, **18**:134-141.

26. Prigge MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE: **Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in *Arabidopsis* development**. *Plant Cell* 2005, **17**:61-76.

27. • Williams L, Grigg SP, Xie M, Christensen S, Fletcher JC: **Regulation of *Arabidopsis* shoot apical meristem and lateral organ formation by microRNA *miR166g* and its *AtHD-ZIP* target genes**. *Development* 2005, **132**:3657-3668.

This paper demonstrates an indirect role for miRNAs in controlling meristem formation by regulating *WUS* transcript levels. The authors show that the meristem-enlargement phenotypes of *jabba-1D* plants were caused by overexpression of *miR166g,* which targeted the transcripts of the class III *AtHD-ZIP* genes *PHB, PHV* and *CNA*. Reduction in the transcript levels of these genes was correlated with a dramatic increase in *WUS* mRNA levels in *jba-1D* SAMs, suggesting that the three class III *AtHD-ZIP* genes restrict SAM activity by downregulating *WUS* transcription.

28. • Jonsson H, Heisler M, Reddy GV, Agrawal V, Gor V, Shapiro BE, Mjolsness E, Meyerowitz EM: **Modeling the organization of the *WUSCHEL* expression domain in the shoot apical meristem**. *Bioinformatics* 2005, **21**:i232-i240.

In vivo confocal microscopy data together with computational modeling techniques were used to dissect the complex interaction networks that lead to the correct positioning of the *WUS* expression domain. The authors' preferred model uses a reaction–diffusion mechanism to produce a pattern of an activator molecule that induces *WUS* expression. A small repressive signal from the L1 cell layer and the stem places the activator peak at the proper location in the OC.